

Claims

1. A bacterial cell which is urease-deficient and which comprises a recombinant nucleic acid molecule encoding a fusion polypeptide comprising (a) at least one domain from a polypeptide, wherein said polypeptide domain is capable of eliciting an immune response in a mammal, and (b) a phagolysosomal escape domain.
2. The cell of claim 1 wherein at least one cellular urease subunit encoding nucleic acid sequence is inactivated.
3. The cell of claim 2 wherein at least the cellular urease C subunit-encoding sequence is inactivated.
4. The cell of claim 1, wherein said phagolysosomal escape domain is a *Listeria* phagolysosomal escape domain.
5. The cell of claim 1, wherein said phagolysosomal domain is encoded by a nucleic acid molecule selected from:
 - (a) a nucleotide sequence comprising nucleotide 211 - 1722 as shown in SEQ ID No.1,
 - (b) a nucleotide sequence which encodes for the same amino acid sequence as the sequence from (a), and
 - (c) a nucleotide sequence hybridizing under stringent conditions with the sequence from (a) or (b).
6. The cell of claim 1, wherein the domain capable of eliciting an immune response is a peptide or polypeptide capable of eliciting MHC class I-restricted CD8 T cell responses.
7. The cell of claim 1 wherein the domain capable of eliciting an immune response is from a *Mycobacterium* polypeptide.

8. The cell of claim 7, wherein the domain capable of eliciting an immune response is selected from the Mycobacterium antigens Ag85B (M.tuberculosis), Ag85B (M.bovis), Ag85A (M.tuberculosis) and ESAT-6 (M.tuberculosis) or an immunogenic fragment thereof.
9. The cell of claim 8, wherein the domain capable of eliciting an immune response is the antigen Ag85B or an immunogenic fragment thereof.
10. The cell of claim 1, wherein the fusion polypeptide is preceded by a signal peptide sequence.
11. The cell of claim 1, wherein a peptide linker is located between the immune response eliciting domain and the phagolysosomal domain.
12. The cell of claim 1, wherein said nucleic acid molecule is operatively linked with an expression control sequence.
13. The cell of claim 12, wherein said expression control sequence is active in said cell.
14. The cell of claim 1 wherein said nucleic acid molecule is located on a vector.
15. The cell of claim 1 which is a Mycobacterium cell.
16. The cell of claim 16 which is a Mycobacterium bovis cell.
17. A bacterial cell which is urease-deficient and which comprises at least one recombinant nucleic acid molecule encoding a phagolysosomal escape peptide or polypeptide.
18. The cell of claim 17, which comprises at least one further recombinant nucleic acid molecule encoding a peptide or polypeptide capable of eliciting an immune response in a mammal.
19. The cell of claim 18 which is a Mycobacterium cell.

20. The cell of claim 19 which is a *Mycobacterium bovis* cell.
- 5 21. The cell of claims 1 or 17, wherein the domain or peptide or polypeptide capable of eliciting an immune response is selected from autoantigens, tumor antigens, virus antigens, parasite antigens, bacterial antigens and immunogenic fragments thereof.
- 10 22. The cell of claims 1 or 17, which is capable of expressing said at least one recombinant nucleic acid molecule.
23. The cell of claim 22, which is capable of secreting a polypeptide encoded by said at least one nucleic acid molecule.
- 15 24. The cell of claims 1 or 23, which has an intracellular persistence in infected macrophages which is equal or less than the intracellular persistence of a native *Mycobacterium* cell.
- 20 25. A pharmaceutical composition comprising as an active agent a cell of claims 1 or 17, optionally together with pharmaceutically acceptable diluents, carriers and adjuvants.
- 25 26. The composition of claim 25, which is a living vaccine suitable for administration to a mucosal surface or via the parenteral route.
27. A method for the preparation of a living vaccine comprising formulating a cell of claims 1 or 17 in a pharmaceutically effective amount with pharmaceutically acceptable diluents, carriers and adjuvants.
- 30 28. A method for preparing a recombinant bacterial cell of claim 1 comprising the steps:
- (i) providing a urease-deficient bacterial cell;
 - (ii) inserting a recombinant nucleic acid molecule into said bacterial cell, said nucleic acid molecule encoding a fusion polypeptide comprising (a) at least one domain from a polypeptide, wherein said domain is capable of eliciting an immune response in a mammal, and (b) a phagolysosomal escape domain, and
- 35

- 24 -

- (iii) cultivating the cell obtained according to (ii) under suitable conditions.

29. The method of claim 28, wherein said cell is a M.bovis cell.

5

30. A method for preparing a recombinant bacterial cell of claim 17 comprising the steps:

- (i) providing a urease-deficient bacterial cell;
- (ii) inserting a recombinant nucleic acid molecule into said bacterial cell, said nucleic acid molecule encoding a phagolysosomal escape peptide or polypeptide and
- (iii) cultivating the cell obtained according to (ii) under suitable conditions.

10

31. The method of claim 30 comprising inserting at least one further recombinant nucleic acid molecule into the bacterial cell, said further recombinant nucleic acid molecule encoding a peptide or polypeptide capable of eliciting an immune response in a mammal.

15

32. The method of claim 28 or 30, wherein the domain or peptide or polypeptide capable of eliciting an immune response is selected from autoantigens, tumor antigens, virus antigens, parasite antigens, bacterial antigens and immunogenic fragments thereof.

20

33. The use of a bacterial cell according to any one of claims 1-24 for the manufacture of a living vaccine.

25

34. The use of claim 33 for the manufacture of a vaccine for immunodeficient subjects.

30

35. The use of claim 34 for the manufacture of a vaccine for subjects suffering from an HIV infection.

36. The use of claim 33 for the manufacture of a tumor vaccine.

35

37. The use of claim 36 for the manufacture of a vaccine against superficial bladder cancer.

- 25 -

38. The use of claim 33 for the manufacture of a vaccine in the veterinary field.